Squamous Cell Carcinoma is not Associated with High Dermal Mast Cell Prevalence in Humans

To the Editor:

Basal cell carcinomas (BCC) and squamous cell carcinomas (SCC), commonly referred to as "nonmelanoma skin cancers", are the most common form of cancers in Caucasian populations (Armstrong and Kricker, 2001). Many genetic and environmental factors contribute to the pathogenesis of these keratinocyte-derived neoplasms, with exposure to ultraviolet radiation (UVR) considered the major causal factor (Rosso *et al*, 1996; English *et al*, 1998). Evidence from epidemiologic studies suggests that the pattern of sun exposure and the dose of solar UVR associated with the development of BCC differs from that of SCC. Intermittent intense sun exposure has been described as one significant risk factor for BCC (Kricker *et al*, 1995; Armstrong and Kricker, 2001), whereas the incidence of SCC increases with age and cumulative sun exposure over a lifetime (Strom and Yamamura, 1997; Armstrong and Kricker, 2001). The process of keratinocyte transformation appears to be UVR dose-dependent in these skin cancers. BCC are believed to arise from mitotic epidermal cells rather than differentiated epithelial keratinocytes as for SCC and, therefore, may require less solar UVR for malignant transformation to occur (Rosso *et al*, 1996).

UVR-induced cutaneous neoplasms are highly immunogenic in that a competent immune response results in tumor rejection, whereas impairment of the immune response by UVR enables the progressive outgrowth of skin cancers (Kripke, 1981). Studies in multiple murine strains have provided evidence of a functional link between mast cell-derived histamine, dermal mast cell prevalence, and susceptibility to UVR-induced systemic immunosuppression (Hart *et al*, 1998). Recently, we reported that Danish patients with a history of sporadic BCC (as the only skin cancer type) have a higher dermal mast cell prevalence in nonsun-exposed buttock skin than age- and sex-matched control subjects (Grimbaldeston *et al*, 2000). Given that the patterns of sun exposure in the epidemiology of the cancer differ between BCC and SCC, in this study we evaluated dermal mast cell prevalence in the buttock skin of Danish patients with SCC.

Twenty Danish patients with a history of histologically confirmed SCC and 20 sex- and age-matched control subjects participated in the study (**Table I**). All volunteers were of Fitzpatrick skin phototypes I, II, and III (Fitzpatrick, 1988). SCC patients were recruited from the Department of Dermatology, Gentofte Hospital (Hellerup, Denmark). Control subjects were selected on the basis of no previous history of malignancy. The exclusion criteria remained the same for this study as in a previous study of BCC (only) patients (Grimbaldeston *et al*, 2000). In all the patients, SCC occurred at sun-exposed skin sites. The majority of the patients (14 of 20) had a single SCC lesion and only one of the 20 SCC patients presented with more than five SCC. The face or scalp were the common sites of SCC localization (16 patients). Three patients had lesions occur on the face together with either the chest or the back. Two women and one man presented with an SCC on the leg. Of the 20 patients, a subgroup of nine also had BCC localized at sun-exposed skin sites with five of these patients previously having at least 10 BCC each at the time of recruitment. Though differing in location and time of appearance, BCC were localized at the same anatomic site as the SCC in five of the nine patients. In this subgroup of nine patients, five people reported that their first skin cancer was a BCC. Interestingly, all five patients had multiple BCC with three SCC patients presenting with greater than 10 BCC. Skin phototype showed no association with this phenomenon.

After informed consent, each participant had a 4 mm punch biopsy of buttock skin taken. All biopsies were coded in Denmark prior to being sent to Australia for quantification in a blinded fashion of dermal mast cell prevalence. The code was retained in Denmark and the SCC or control status for each biopsy revealed only after analysis of the cohort had been completed. Sections (4 μm) were cut, immunohistochemically stained with an anti-histamine antibody (Chemicon, Temecula, CA), and dermal mast cell prevalence quantified using computer-generated image analysis as previously described (Grimbaldeston *et al*, 2000). In brief, depending on the thickness of the dermis (papillary + reticular layer), 173–490 consecutive fixed fields were captured in each section analyzed. Two consecutive sections from each biopsy were assessed for dermal mast cell prevalence, and the mean obtained. Fields were captured horizontally parallel to the epidermis followed by the papillary and reticular layers of the dermis using a $\times 40$ microscope objective and a $\times 2.5$ camera eyepiece. Mast cells (as determined by histamine-staining cells) per fixed field of 140 μm width were counted (but expressed per mm^2); the coefficient of variation for measurement of mean dermal mast cell prevalence for 173–490 fields was $<15\%$. A study of interbiopsy variability in dermal mast cell numbers from a single donor ($n = 6$ buttock skin biopsies) over 6 mo demonstrated that mast cell prevalence was reproducible over this period of time (Grimbaldeston *et al*, 2000). Quantification of all biopsies was performed by the same investigator.

In contrast to our previous finding in Danish BCC patients (Grimbaldeston *et al*, 2000), there was no significant difference in dermal mast cell prevalence between SCC patients and control subjects ($p = 0.978$, Mann–Whitney U; **Fig 1**).

The fact that nine of our 20 Danish SCC patients also had a history of BCC and mast cell prevalence equivalent to controls (**Fig 1**) requires comment as we have established that patients with BCC (only) had a significantly higher median dermal mast cell density than controls. We propose that high dermal mast cell prevalence in nonsun-exposed skin is only one susceptibility factor (assuming sufficient UVR) for the development of sporadic BCC [nodular and superficial BCC types (Bastiaens *et al*, 1998) could not be differentiated due to removal by curettage]. BCC patients with a lower dermal mast cell prevalence may have other etiologic factors associated with the development of their BCC, such as lesional mutations in the *PTCH* tumor suppressor gene (Gailani *et al*, 1996). In sporadic BCC 30–40% have "UV signature" *PTCH* alterations (Lacour, 2002). To differentiate further, mutations in the *PTCH* tumor suppressor gene have been found

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Table I. Sex, age, and skin phototype in control subjects and SCC patients

	Sex		Age (y)	Skin phototype			Number of SCC		
Group	M	F	mean (range)	I	II	III	1	2–5	6–10
Control	10	10	69 (53–84)	1	14	5	0	0	0
SCC	12	8	72 (56–84)	1	15	4	15	4	1

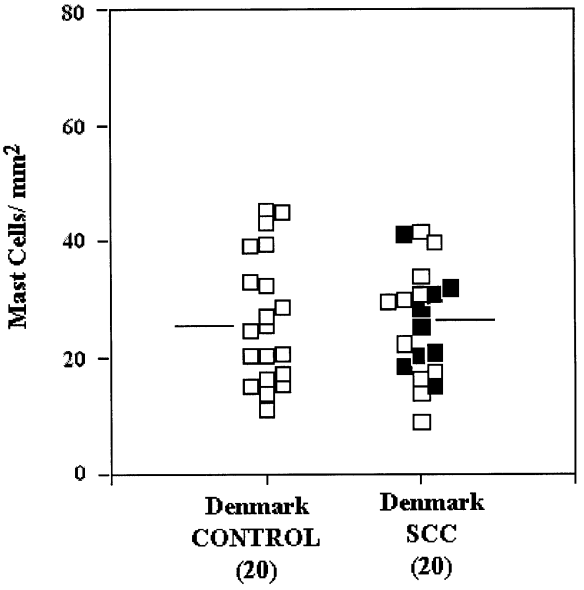

Fig 1. Mast cells per mm^2 dermal area in buttock biopsies from Danish SCC patients and control subjects. □ represents the mean mast cell number expressed per fixed field area for 346–980 measurements. SCC (n = 20 patients); control (n = 20 subjects). Median values are 27 mast cells per mm^2 (range = 9–42) and 25 mast cells per mm^2 (range = 11–45) for SCC patients and control subjects, respectively. There was no significant difference in the prevalence of dermal mast cells between SCC patients and control subjects (p = 0.978, Mann–Whitney U). SCC patients (n = 9) that have also presented clinically with BCC.

in BCC lesions from patients with SCC and a history of multiple sporadic BCC (Ping *et al*, 2001). Interestingly, five of the nine Danish SCC patients had multiple (>10) BCC and five of the nine patients also reported that the first cutaneous cancer to appear was a BCC. Heterozygous *PTCH*$^{+/-}$ mice exposed to a 3 minimal erythema dose of UV three times per week for 12 mo all presented with multiple BCC after 3 mo of age followed by the appearance of SCC 6 mo later (Aszterbaum *et al*, 1999). This pattern of carcinogenesis resembles the clinical presentations of the Danish SCC/BCC patients in our study.

The action spectrum, timing, and doses of UVR involved in the development of SCC and BCC in humans are yet to be fully elucidated but may differ between these keratinocyte-derived cancers. Mechanisms modulating the immune response and mutations in proto-oncogenes or suppressor genes may also vary between subtype. We hypothesize that dermal mast cells may function in humans, as in mice, by initiating immune suppression and when present at comparatively high prevalence, enabling a permissive environment for the development of a subset of BCC. In contrast, development of SCC and BCC in patients with multiple SCC may be supported by additional immunomodulatory mechanisms. UV-specific cyclobutane pyrimidine dimers DNA lesions in the *p53* gene have been found in over 90% of SCC and in only 50% of BCC (Ananthaswamy *et al*, 1998). Studies in murine strains suggest that cyclobutane pyrimidine dimers may play a part in UVR-induced immunomodulation (Yarosh *et al*, 2000) by upregulation of tumor necrosis factor-α gene transcription and protein expression (Kibitel *et al*, 1998), a cytokine that has been linked with susceptibility to UVR-induced local immunosuppression. Ultimately, identification of the mechanism(s) that signal immune suppression may provide a greater understanding of the etiology and pathogenesis of keratinocyte-derived skin cancer that may present possibilities for intervention and have implications for the development of desirable sunscreen properties.

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